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Authors: Jacobien M. van Peer, Dorien Enter, Henk van Steenbergen, Philip Spinhoven, Karin Roelofs

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Exogenous Testosterone Affects Early Threat Processing in Socially Anxious and Healthy Women

Jacobien M. van Peer PhD[a,b,1*],Dorien Enter PhD[a,b,c,1], Henk van Steenbergen PhD[c,d], Philip Spinhoven PhD[c,e], Karin Roelofs PhD[a,b]

[a] Radboud University Nijmegen, Behavioural Science Institute (BSI), Nijmegen, Montessorilaan 3, PO Box 9104 ,6500 HE Nijmegen, The Netherlands. d.enter@psych.ru.nl

[b] Donders Institute for Brain, Cognition and Behaviour, PO Box 9101, 6500 HB Nijmegen, The Netherlands. k.roelofs@donders.ru.nl

[c] Leiden University, Institute of Psychology, Leiden, Wassenaarseweg 52, 2333 AK Leiden, The Netherlands. HvanSteenbergen@fsw.leidenuniv.nl; Spinhoven@fsw.leidenuniv.nl

[d] Leiden Institute for Brain and Cognition, c/o LUMC, PO Box 9600, 2300 RC Leiden, The Netherlands

[e] Leiden University Medical Center, Department of Psychiatry, PO Box 9600, 2300 RC Leiden, The Netherlands

[1] shared first author

*corresponding author:

Jacobien van Peer

Radboud University Nijmegen, Behavioural Science Institute (BSI), Montessorilaan 3, PO Box 9104, 6500 HE Nijmegen, The Netherlands. Tel: +31 (0)24-3611593, Fax: +31 (0)24-3615594, j.vanpeer@psych.ru.nl

Highlights

- We applied single dose testosterone administration in a placebo-controlled design.
- Testosterone selectively affected early automatic emotional face processing.
- The results did not differ between socially anxious and non-anxious women.
- The findings support social motivational theories of testosterone.

Abstract

Testosterone plays an important role in social threat processing. Recent evidence suggests that testosterone administration has socially anxiolytic effects, but it remains unknown whether this involves early vigilance or later, more sustained, processing-stages. We investigated the acute effects of testosterone administration on social threat processing in 19 female patients with Social Anxiety Disorder (SAD) and 19 healthy controls. Event-related potentials (ERPs) were recorded during an emotional Stroop task with subliminally presented faces. Testosterone induced qualitative changes in early ERPs (<200 ms after stimulus onset) in both groups. An initial testosterone-induced spatial shift reflected a change in the basic processing (N170/VPP) of neutral faces, which was followed by a shift for angry faces suggesting a decrease in early threat bias. These findings suggest that testosterone specifically affects early automatic social information processing. The decreased attentional bias for angry faces explains how testosterone can decrease threat avoidance, which is particularly relevant for SAD.

Keywords: Testosterone; Social Anxiety; Event-related potentials; Emotional Stroop; Social Threat

1. Introduction

Testosterone has an important role in the regulation of social motivational behavior. A surge in testosterone has anxiolytic effects and facilitates social dominance and approach behavior in socially challenging situations (Archer, 2006; Bos, Panksepp, Bluthe, & van Honk, 2012; Terburg & Van Honk, 2013). Especially an angry looking face with direct gaze is perceived as a signal of social threat, as it can signal an impending aggressive encounter (Öhman, 1986). In line with this notion, recent single dose administration studies showed that testosterone promotes approach action tendencies to angry faces on a social approach-avoidance task (Enter, Spinhoven, & Roelofs, 2014, 2016). In addition, it facilitated socially dominant gaze behavior as indicated by increased fixation to the eyes of angry faces (Enter, Terburg, Harrewijn, Spinhoven, & Roelofs, 2016; Terburg et al., 2016; Terburg, Aarts, & van Honk, 2012).

At the neural level, testosterone has been found to enhance the reactivity of the amygdala towards angry facial expressions (Goetz et al., 2014; Hermans, Ramsey, & van Honk, 2008), and to reduce its connectivity with circuits involving the orbitofrontal or prefrontal cortex, thalamus, brainstem, and striatum (van Wingen, Mattern, Verkes, Buitelaar, & Fernandez, 2010; Volman, Toni, Verhagen, & Roelofs, 2011). Furthermore, using a social approach-avoidance task, in which angry and happy faces have to be approached or avoided by pulling or pushing a joystick, Radke et al. (2015) showed that testosterone *increased* amygdala responses specifically during *approach* of angry faces, but *decreased* amygdala responses during angry face *avoidance*, suggesting that testosterone modulates social threat processing in a motivation-specific manner. However, little is known about the temporal dynamics of these effects, and it remains unknown whether they involve early vigilance or later, more sustained, stages of social threat processing. Gaining insight into these processes would be of particular interest for Social Anxiety Disorder (SAD), as this frequent and persistent disorder is characterized by increased early automatic vigilance and biased goal-directed processing of social threat (for reviews see Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007; Gilboa-Schechtman & Shachar-

Lavie, 2013; Staugaard, 2010) as well as by decreased salivary levels of basal testosterone (Giltay et al., 2012).

In the current study we therefore investigate the effects of testosterone on social threat processing in participants with SAD and healthy participants, using temporally fine-grained recordings of the event-related brain potentials (ERPs) during an emotional Stroop task with subliminally presented (i.e., backward masked) angry, happy and neutral faces. We used the masked version of this paradigm, as previous studies suggested that effects of social anxiety (Putman, Hermans, & van Honk, 2004) and hormonal manipulations (cortisol: Van Honk et al., 1998; van Peer, Spinhoven, & Roelofs, 2010, and testosterone: Van Honk et al., 2000; Wirth & Schultheiss, 2007) are stronger in this version than in the unmasked version. We performed a spatiotemporal clustering analysis (Brunet, Murray, & Michel, 2011; Murray, Brunet, & Michel, 2008) on the ERPs, as this method has several advantages compared to conventional ERP amplitude analyses. Most importantly, it can tease apart the following two ERP effects: 1) topographic modulations, which reflect a change in neural sources, indicating the activation of different cognitive processes (a *qualitative* change in processing), and 2) amplitude modulations, which, in absence of a concurrent topographic modulation, reflect increases or decreases in response strength of a common cognitive process (a *quantitative* change in processing) (see e.g., Murray et al., 2008; Pourtois, Delplanque, Michel, & Vuilleumier, 2008). Furthermore, this method may be more sensitive in detecting differences between groups or task conditions (Murray et al., 2008), as it includes the full range (instead of only a limited number) of channels and time windows, and it can detect topographic changes even when amplitude is low. Particularly for pharmacological interventions like testosterone, which modulates multiple parts of the emotion circuitry (see e.g., Bos et al., 2012; van Wingen et al., 2010), effects are unlikely to be bound to single ERP peaks.

Previous ERP studies have shown increased amplitudes for subliminally presented angry (compared to neutral) faces especially on early components such as the frontocentral P2 or VPP

(van Peer et al., 2010), the N2 (Balconi & Lucchiari, 2005, 2007), and the EPN (Mühlberger et al., 2009). Some studies with supraliminal stimuli suggest that this effect may be amplified in socially anxious compared to non-anxious participants (e.g., P1 amplitude: Mueller et al., 2009; Hagemann, Straube, & Schulz, 2016; N170 amplitude: Kolassa & Miltner, 2006; Wieser, Pauli, Reicherts, & Mühlberger, 2010; see also P3/LPP amplitude: Moser, Huppert, Duval, & Simons, 2008; Hagemann et al., 2016). Other studies, however, reported increased early amplitudes in high socially anxious participants irrespective of emotion (e.g., P1: Kolassa et al., 2009; Peschard, Philippot, Joassin, & Rossignol, 2013; Rossignol, Campanella, Bissot, & Philippot, 2013; Rossignol, Philippot, Bissot, Rigoulot, & Campanella, 2012), suggesting a general early hypervigilance to face stimuli. Overall electrophysiological evidence for hypervigilance to social threat in social anxiety is still inconsistent (cf., Kanai, Nittono, Kubo, Sasaki-Aoki, & Iwanaga, 2012; Mühlberger et al., 2009; Wangelin, Bradley, Kastner, & Lang, 2012) and findings may depend on task conditions and choice of specific ERPs (see Schulz, Mothes-Lasch, & Straube, 2013 for a review).

Based on these ERP studies, we expected to find increased processing of angry faces mainly in the early (<250 ms) time window. Most importantly, based on the social-anxiolytic and approach-promoting effects of testosterone (Archer, 2006; Terburg & Van Honk, 2013) we expected that testosterone administration compared to placebo would reduce processing of angry versus neutral and happy faces, particularly in SAD patients who are characterized by a social threat bias as well as lower endogenous testosterone levels. Finally, based on behavioral findings suggesting that effects of testosterone are most pronounced for preconscious processing of threat (Van Honk et al., 2000) we hypothesized that these effects may be predominantly manifested in the early processing stages.

2. Materials and Methods

2.1. Participants

Participant characteristics are presented in Table 1. Participants for the Social Anxiety Disorder (SAD) group were recruited from outpatient anxiety departments of mental health centers, through advertisements on the internet, and in local newspapers. Inclusion criterion was a total score of > 60 on the Liebowitz Social Anxiety Scale (Liebowitz, 1987; Rytwinski et al., 2009). In addition these participants were screened with the Mini International Neuropsychiatric Interview script (M.I.N.I.; Lecubier et al., 1997) to verify the DSM-IV diagnosis of generalized Social Anxiety Disorder. One participant (LSAS score 56) scored just below the LSAS cutoff but was included as she did fulfill the DSM-IV diagnostic criteria. Healthy control (HC) participants were recruited via advertisements in community centers, on the internet, and in local newspapers. Only female participants were included, because the parameters (e.g., dose and time course) for inducing neurophysiological effects in men with a single dose administration of testosterone are as yet unknown (Tuiten et al., 2000). Both women using single-phase contraceptives and normally cycling women participated in the study. All participants had normal or corrected-to-normal vision. Exclusion criteria were age < 18 and > 50, use of (psychotropic) medication, somatic illnesses, neurological conditions, recent or past psychiatric problems (HC group only), psychotic disorder, current comorbid diagnosis of mood or anxiety disorders other than SAD (SAD group only), history of head injury, left-handedness, peri- or postmenopause, and pregnancy or breast feeding. Initially, 24 participants were included in each group. However, as these groups differed significantly in age ($F(1,46) = 12.59$, $p = .001$, $\eta^2 = 0.21$), and there is no appropriate method to statistically control for such an effect in the analyses (Miller & Chapman, 2001), a subset of 19 SAD and 19 HC participants (age $F(1,36) = 2.78$, $p = .104$, $\eta^2 = 0.07$, see table 1) was selected on basis of matching for age (Field, 2009; see also Enter, Terburg, et al., 2016). Thirteen of the 19 SAD participants met full DSM-IV criteria for generalized SAD at the time of testing; the other five had subsyndromal SAD (i.e., they fulfilled all criteria at the telephone screening but the symptoms did no longer lead to significant burden in social or occupational

functioning [DSM-IV criterion IV] at time of testing). All participants provided written informed consent, and received financial compensation. The study was approved by the Medical Ethics Committee of the Leiden University Medical Centre, and was in accordance with the declaration of Helsinki.

2.2. Testosterone Administration

In a double-blind, randomized, placebo-controlled, cross-over design participants received a single dose of 0.5 mg testosterone suspended in a clear solution (0.5 ml) with 0.5 mg hydroxypropyl-beta-cyclodextrin, 0.005 ml ethanol 96%, and distilled water. The matched placebo contained the same ingredients, except for the testosterone. Participants were asked to hold the liquid under their tongue for 60 s. The order of placebo and testosterone administration was counterbalanced across subjects and groups (see Table 1). In females, this dose yields a sharp increase of 20–25 nmol/l in plasma testosterone levels within 15 min, which declines to baseline levels within the next 90 min (van Rooij et al., 2012). Previous research applying this procedure (see Bos et al., 2012 for a review; Eisenegger, Naef, Snozzi, Heinrichs, & Fehr, 2010; Enter et al., 2014; Enter, Spinhoven, et al., 2016; Enter, Terburg, et al., 2016; Tuiten et al., 2000) has convincingly shown consistent psychophysiological and behavioral effects approximately 4–6 h after administration, therefore this time interval was also applied in the current study

2.3. Procedure

Participants were tested individually at two identical testing sessions with two days in between. Testing sessions started at either 09:30 or 13:30, on the same time at both sessions. Five hours after testosterone or placebo intake, participants were seated in a dimly lit and sound attenuated room, where they performed the Emotional Stroop Task while EEG was recorded simultaneously. Between testosterone or placebo intake and the Emotional Stroop task, participants had a two

hour resting period in a private recreation room (without television or internet access) where they could read and rest, followed by a standard lunch and several unrelated tasks of which the results will be reported elsewhere (i.a., Enter et al., 2014; Enter, Spinhoven, et al., 2016; Enter, Terburg, et al., 2016). Participants were not informed about the expectancies regarding testosterone in this study. Furthermore, after completion of the two sessions, participants had to indicate in which session they thought to have had testosterone or placebo. Statistical analysis of these choices compared to the actual treatment (X^2 (1, n= 38) = 1.69, p= .194) confirmed that participants could not reliably predict when they had received testosterone, and thus were blind with regard to the treatment conditions.

2.4. Emotional Stroop Task

Face stimuli were selected from the Pictures of Facial Affect (Ekman & Friesen, 1976) and the Karolinska Directed Emotional Faces (Lundqvist, Flykt, & Öhman, 1998) databases. Angry, happy, and neutral facial expressions were taken from the same model (four male and four female models), cut out in an oval shape to remove distracting features, gray-scaled, and presented with a red, green or blue filter on a black background. Masking stimuli consisted of oval configurations of randomly cut and reassembled fragments of face stimuli (Van Honk et al., 1998). The total stimulus set consisted of 72 target face stimuli (8 actors x 3 expressions x 3 colors) and 6 masks (2 versions x 3 colors) (see also van Peer et al., 2010). Stimulus presentation and response logging were controlled using E-prime software, a Serial Response Box (Psychology Software Tools, inc.) and a custom-made manual response box.

Participants started with a practice block of nine trials in which only masks were presented. Next, they completed the 72 randomized trials. Each trial started with a 750 ms fixation cross, followed by a very brief (16.7 ms, 2 frames at 120 Hz) exposure to a target face, which was replaced by a mask of the same color. Participants were instructed to categorize this color as fast

as possible by pressing the corresponding button, and their response triggered offset of the masks. New trials started after a random inter-trial interval of 2-4 s.

Incorrect responses were excluded from the analyses. Reaction time (RT) outliers were filtered using a < 200 and > 1300 ms cut-off, and subsequently all RTs exceeding 2.5 SD from the individual participants' mean were removed. These trials were also excluded from the EEG analyses. Of the remaining latencies (HC 94%, SAD 95%), the means were calculated per group and condition and log-transformed because of a skewed distribution.

To determine whether participants were capable of consciously perceiving the masked facial expressions, they were asked to complete an awareness check at the end of the second testing session (see Supplementary Material).

2.5. Electrophysiological Recording and Analyses

The EEG was recorded at 512 Hz with an Active-Two system (BioSemi, Amsterdam, The Netherlands) from 32 active electrodes referenced to an active common mode sense and with a passive driven right leg ground electrode. All electrodes were mounted in an elastic cap and distributed over the head surface according to the international extended 10–20 system. To ensure consistency of electrode placement, the nasion-inion and intra-auricular distances were measured and documented during the first session, the center of the electrode cap (Cz) was positioned halfway between each of these two distances, and the same cap and distances were used during the second session. Horizontal and vertical EOGs were recorded using four bipolar electrodes placed on the outer canthi of the eyes and in the inferior and superior areas of the left orbit. Signals were processed offline using Brain Vision Analyzer software (Version 2.1). Bad EEG channels were interpolated using a topographic interpolation as recommended by Brunet et al. (2011), with a maximum of three channels (10%) for each individual data set (M = 0.29, SD = 0.57). Subsequently, EEG data were re-referenced to an average reference, filtered with a 0.1-

Hz high-pass filter (24 db/oct), and epoched from 200 ms before until 800 ms after stimulus onset. After baseline correction on the pre-stimulus interval, data were corrected for the effects of eye blinks and eye movements using a standard procedure (Gratton, Coles, & Donchin, 1983), and epochs containing artifacts (amplitude values > 100 or < -100 μV, a difference of 150 μV between the lowest and the highest amplitude within 200 ms, a difference > 75 μV between two subsequent sampling points, or a period of 100 ms with activity < 0.50 μV), were removed. Finally, data were averaged to individual ERPs for each facial expression type (happy, angry, and neutral, *M* = 21.5, *SD* = 1.7 trials per category), excluding trials with incorrect responses or outlier RTs (see RT analyses in section 2.4). See Supplementary Material for an overview of the number of remaining trials and grand average ERPs per group and condition. The data of two participants (one HC and one SAD) were excluded because of an excessive number of artifacts (< 15 trials left in one or more conditions), resulting in 36 participants (18 HC, 18 SAD) for the statistical analyses.

2.5.1. Spatiotemporal clustering

Spatiotemporal clustering analysis was performed using the Cartool software by Denis Brunet (version 3.53, brainmapping.unige.ch/cartool, Brunet et al., 2011) to identify dominant topographic maps of the scalp electric field in the grand-averaged ERP data, and to compare the expression of these maps over time and across groups and experimental conditions. Each topographic map, which usually remains stable for several tens of milliseconds, has been proposed to reflect a period of coherent synchronized activation of large-scale neural networks (functional microstate, see e.g., Lehmann, 1987). Different topographic maps reflect the activation of different neural networks or microstates (e.g., Michel, Seeck, & Landis, 1999), and the typical finding of a sequence of different maps is suggested to represent successive information processing steps (see e.g., Lehmann, 1987). Segmentation of the post-stimulus time window was performed using the Topographic - Atomize and Agglomerate Hierarchical Clustering (T-AAHC) procedure, with rejection of segments smaller than 4 time frames (~ 8 ms) and merging of clusters

that correlated above 0.92 (temporal smoothing, see Brunet et al., 2011). The optimal spatiotemporal solution explaining the whole data set was determined by using an objective cross-validation (CV) and modified Krzanowski-Lai (KL) criterion (Pascual-Marqui, Michel, & Lehmann, 1995; for explanation see Brunet et al., 2011; Murray et al., 2008). The resulting dominant topographic maps (see Figure 1) were fitted back, in separate time intervals, to each time frame of the individual average ERPs, using a noncompetitive spatial fitting procedure with rejection of segments < 4 time frames. This procedure provides a quantitative value (the global explained variance [GEV], reflecting the total squared spatial correlation between the maps and the data, a measure of the goodness of fit) for the representation of each map across participants and conditions. The maps were fitted within four different time intervals, based on the conventional time windows of the corresponding ERP components (80-120 ms [P1, Map #2]; 120-200 ms [N170/VPP, Map #3 and #4]; 180-300 ms [P2, Map #5 and #6]; 275-800 ms [P3/LPP, Map#7 to #10]). The first map (Map #1, 0-80 ms) was excluded from the analyses, as the corresponding ERP component (C1) is known to be exogenous and pre-attentive (Pratt, 2011).

2.5.2. Global Field Power

Changes in neural response strength were determined by calculating the global field power (GFP) with Cartool (Brunet et al., 2011). GFP is equivalent to the standard deviation of the scalp electric field, with large values corresponding to moments of high synchronized neural activity (e.g., Lehmann, 1987). For each participant and condition, mean GFP was calculated in three time intervals that were symmetrically centered around the peaks in the grand average (see Figure 3): 80-120 ms, 125-185 ms, and 215-275 ms. As for the spatiotemporal results, the first peak (~70 ms), corresponding to the C1 component, was excluded from the analyses.

2.6. Statistical Analyses

All data were analyzed with the Statistical Package for the Social Sciences (SPSS 21) using repeated-measures analyses of variance (ANOVA) with Treatment (placebo, testosterone) and Valence (angry, happy, neutral) as within-subject factors, and Group (HC, SAD) as between-subjects factor. Separate ANOVAs were performed on each time interval of the spatiotemporal clustering and GFP data. The spatiotemporal clustering analyses included the additional factor Map in case more than one map was present in the respective time interval. Significant interactions were followed by tests of simple effects with rm ANOVA's at each level of the relevant factors, to determine the nature of the interaction. Finally, several control analyses were conducted, first to check that the findings were not influenced by awareness of the subliminal stimuli, and second to check for the influence of possible confounding factors such as order of treatment, time of testing, or use of contraception. The results of these analyses are reported in the Supplementary Material, and did not differ notably from the results reported below. All statistical analyses used a two-tailed alpha of .05. Effect sizes of significant results are reported as the proportion of explained variance (partial eta squared [η_p^2]).

3. Results

3.1. Behavioral Results

The response latencies for each group and condition are presented in Table 2. The statistical analysis revealed a significant main effect of Valence, $F(2,72) = 3.34$, $p = .041$, $\eta_p^2 = .09$. Post hoc pairwise comparisons indicated that the response latencies were significantly slower for angry, $F(1,36) = 4.64$, $p = .038$, $\eta_p^2 = 0.11$, and happy, $F(1,36) = 4.90$, $p = .033$, $\eta_p^2 = 0.12$, compared to neutral faces, suggesting an interference effect for emotional faces. Response latencies for happy and angry faces did not differ significantly, $F(1,36) = 0.12$, $p = .732$. When the two participants with

missing EEG data were excluded, the main effect of Valence was a trend, $F(2,68) = 2.95$, $p = .059$, $\eta_p^2 = .08$, and the pairwise comparisons with neutral faces ($M = 445$, $SEM = 9$) remained significant for both angry ($M = 453$, $SEM = 9$), $F(1,34) = 4.16$, $p = .049$, $\eta_p^2 = .11$, and happy faces ($M = 455$, $SEM = 11$), $F(1,34) = 4.32$, $p = .045$, $\eta_p^2 = .11$. In contrast to the EEG findings, the behavioral results showed no significant effects of Treatment or Group (all $ps > .05$).

3.2. Spatiotemporal Clustering

The spatiotemporal clustering procedure revealed ten distinct dominant field topographies (maps) that together explained 92% of the total ERP variance (see Figure 1A and B). Results of the subsequent spatial fitting procedure, reflecting the representation of these maps (in terms of global explained variance, GEV) across participants, conditions, and time, are reported below. We report only statistical results including interactions of Treatment or Group with Valence and Map, as these reflect the effects of interest of the current study: Testosterone- or SAD-related topographic (i.e., qualitative) differences in emotion-related face processing. See Supplementary Material for additional results.

3.2.1. Effects of testosterone administration

In line with the hypothesis that testosterone affects early processing of emotional faces, the rm ANOVA of GEV in the N170/VPP time interval (120-200 ms post-stimulus, Maps #3 and #4) showed a significant interaction of Treatment x Valence x Map, $F(2,68) = 5.87$, $p = .004$, $\eta_p^2 = 0.147$ (see Figure 2). Follow-up analyses (i.e., rm ANOVA Group x Treatment x Valence per map) revealed that the Treatment x Valence interaction was significant for both maps (Map #3, $F(2,68) = 3.61$, $p = .032$, $\eta_p^2 = .096$; Map #4, $F(2,68) = 4.09$, $p = .021$, $\eta_p^2 = .107$). The first map (Map #3) reflects the typical spatiotemporal pattern of the N170/VPP component (see Figure 1C). Follow-up tests per Valence for this map showed a significant effect of Treatment, reflecting a decrease in GEV after testosterone administration, compared to placebo, for neutral faces,

$F(1,34) = 6.84$, $p=.013$, $\eta_p^2 = 0.167$, and a trend in the same direction for happy faces, $F(1,34) = 3.91$, $p=.056$, $\eta_p^2 = .103$, but not for angry faces, $F(1,34) = 3.15$, $p=.579$, $\eta_p^2 = .009$. This finding suggests that testosterone administration resulted in a reduction of the representation (goodness of fit) of the N170/VPP pattern during neutral (and happy) but not angry face processing. The effect of Valence was not significant in either Treatment condition (placebo $F(2,68) = 1.57$, $p=.215$; Testosterone $F(2,68) = 1.99$, $p=.144$).

The second map in this time interval (Map #4), which has a relatively more positive occipito-parietal topography (see Figure 1C), reflects the activation of a different set of neural sources. In contrast to Map #3, post hoc analyses of the significant Treatment x Valence interaction for this map showed a significant effect of Valence in the placebo condition, $F(2,68) = 3.61$, $p=.050$, epsilon = 0.69, $\eta_p^2 = 0.10$, but not in the testosterone condition, $F(2,68) = 0.93$, $p=.398$. In the placebo condition, GEV was significantly increased for angry compared to happy faces, $F(1,34) = 7.21$, $p=.011$, $\eta_p^2 = 0.175$, whereas the differences between angry and neutral or happy and neutral faces were both nonsignificant (both ps>.05). Furthermore, follow-up tests by Valence showed that compared to placebo, testosterone administration selectively reduced the GEV for angry faces, $F(1,34) = 7.12$, $p=.012$, $\eta_p^2 = .173$. The effect of Treatment was not significant for happy or neutral faces (both ps>.05). These findings suggest that this second configuration of neural activity (map #4) was activated mainly during the processing of angry faces in the placebo condition, possibly reflecting an initial threat bias, which disappeared after testosterone administration.

No significant interactions including Treatment and Valence were present in any of the other time intervals (80-120 ms, 180-300 ms, or 275-800 ms), suggesting that testosterone-induced qualitative changes in emotion-related face processing were limited to the N170/VPP processing stage.

3.2.2. Effects of SAD

No significant effects involving the factor Group were present in any of the time windows (all *p*s >.05), suggesting that there were no significant qualitative differences in emotion-related face processing between SAD and HC participants.

3.3. Global Field Power

Global Field Power was analyzed to test for differences in response amplitude between groups and conditions, independent of changes in topography. The means for the time intervals of interest are presented in Table 3. The results showed a trend towards a main effect of Group in the first time interval (80-120 ms), $F(1,34) = 4.00$, $p=.054$, $\eta_p^2 = 0.105$, suggesting that the P1 amplitude tended to be increased for SAD compared to HC participants, see Figure 3. No other effects reached significance, in any of the time intervals (all *p*s >.05).

4. Discussion

In this study we investigated the effects of single dose testosterone administration on social threat processing in socially anxious and non-anxious participants, by recording event-related brain potentials (ERPs) during an emotional Stroop task with subliminally presented angry, happy and neutral faces. The spatiotemporal clustering results show that testosterone selectively affects the early automatic processing of emotional faces, suggesting a reduced initial processing of neutral faces followed by a decreased processing bias for angry faces, while leaving later processes (>200 ms) unaffected. These effects occurred independent of clinical status of the participants and will be discussed in detail below.

The time interval 120-200 ms post-stimulus showed two distinct topographic patterns, which reflect the activity of different neural populations (e.g., Michel et al., 1999) and indicate the presence of two consecutive information processing steps(microstates, e.g., Lehmann, 1987).

Both of these were affected by testosterone, but in a different manner. The spatiotemporal characteristics of the first pattern correspond to the N170/VPP ERP complex, which is considered to reflect the early stages of face perception and basic-level categorization (see e.g., Blau, Maurer, Tottenham, & McCandliss, 2007a; Conty, Dezecache, Hugueville, & Grèzes, 2012; Rossion & Jacques, 2011). This pattern showed a significant reduction in global explained variance after testosterone administration, compared to placebo, for neutral faces, which suggests that testosterone changes the neural sources involved in the initial face perception process for neutral but not for angry faces.

The second topographic pattern (map #4), with a relatively more positive occipito-parietal topography, reflects the subsequent activation of a different cognitive process. This finding is consistent with evidence suggesting that the N170/VPP complex on the scalp reflects the activity of multiple neural sources overlapping in time, and represents the intermixed processing of several sources of facial information, including not only basic structural but also high-level (e.g. expression, identity) features (Hinojosa, Mercado, & Carretie, 2015; Rossion & Jacques, 2011). These processes may not be distinguishable with traditional ERP amplitude measures, but can be teased apart by investigating topographic modulations (see e.g., Murray et al., 2008). Moreover, this second pattern was more pronounced during the processing of angry (compared to happy) faces in the placebo condition, suggesting that it may reflect an early processing bias for social threat. This is consistent with recent studies suggesting that the N170 time window is differentially sensitive to emotional expressions, and most strongly responds to angry faces (see Hinojosa et al., 2015 for a meta-analysis). Some authors (e.g., Del Zotto & Pegna, 2015; Hinojosa et al., 2015) have suggested that this emotional modulation of the N170 may reflect emotional attention processes driven by the amygdala (see also Conty et al., 2012), to allow for rapid responses to threat. Most interestingly, this angry face advantage disappeared after testosterone administration, which suggests that testosterone eliminated this early processing bias for social threat.

Taken together, our results reveal that testosterone differentially affects the processing of threatening (angry) and non-threatening (neutral and happy) face stimuli in very early processing stages. These findings may reflect the neural processes underlying previous behavioral findings of threat-specific effects of testosterone (for a review see Bos et al., 2012). In particular, the testosterone-induced reduction in early threat bias may explain previous behavioral findings of anxiolytic-like, or approach promoting, effects. For example, studies in healthy participants have shown that testosterone administration reduced the attentional bias to fearful faces (Van Honk, Peper, & Schutter, 2005), as well as the conscious recognition (Van Honk & Schutter, 2007), behavioral avoidance (Enter et al., 2014), and gaze aversion (Terburg et al., 2012) of angry faces. Testosterone was also found to decrease gaze avoidance (Enter, Terburg, et al., 2016) and to promote behavioral approach (Enter, Spinhoven, et al., 2016) in patients with SAD. In apparent contrast to these behavioral findings, several fMRI studies have shown that testosterone *increased* amygdala responses during the processing of angry faces (Goetz et al., 2014; Hermans et al., 2008). However, using an approach-avoidance task, Radke et al. (2015) showed that such increased amygdala responses were specifically related to threat approach behavior, while testosterone *decreased* amygdala responses during threat avoidance. Thus, the effects of testosterone on amygdala activity appear to be context-dependent. Based on these findings, and in line with the behavioral findings described above, it was suggested that, by modulating amygdala responses, testosterone biases humans toward approach, and away from avoidance, of social threat (Radke et al., 2015; see also Enter et al., 2014; Enter, Spinhoven, et al., 2016). Our findings are in line with such an approach promoting or threat reducing effect of testosterone, and suggest that testosterone-induced modulations of neural activity may happen already during the earliest stages of angry face processing. More research is needed to directly investigate the relation between spatial and temporal effects of testosterone on neural activity (e.g., combined fMRI and EEG), and how these relate to behavior, including the role of motivational context.

In addition to these effects of testosterone, our results showed a marginally significant group difference in Global Field Power, reflecting stronger early (80-120 ms post stimulus) neural responses for SAD compared to HC participants. This is consistent with the increased P1 amplitude in high socially anxious participants reported in some previous ERP studies (Peschard et al., 2013; Rossignol et al., 2013, 2012), which has been suggested to reflect a general hypervigilance to face stimuli. However, we did not find group differences in the processing of angry faces (see also Mühlberger et al., 2009, but cf. Kolassa & Miltner, 2006; Schulz et al., 2013), or enhanced amplitudes for angry compared to neutral or happy faces (cf., Balconi & Lucchiari, 2005, 2007; van Peer et al., 2010). Overall, ERP evidence for hypervigilance to social threat in social anxiety is still rather inconsistent (see Schulz et al., 2013 for a review). This may be partly due to limitations of the conventional ERP analysis method, such as the inability to differentiate between amplitude and topographic changes (quantitative and qualitative changes, see e.g., Murray et al., 2008), a strong reference-dependence (Murray et al., 2008; Rellecke, Sommer, & Schacht, 2012), and a small (and often different) selection of electrodes and time windows that are included in the analyses. These limitations can be overcome by using reference-free multichannel spatiotemporal clustering methods (e.g., Michel et al., 1999; Murray et al., 2008; Pourtois et al., 2008) as was done in the present study. It would be recommendable for future research to include similar measures to produce more robust findings and further elucidate the nature of social threat processing in SAD and HC.

On a behavioral level, color naming latencies were significantly slower for angry and happy compared to neutral faces, reflecting the typical Emotional Stroop interference-effect (see e.g., Bar-Haim et al., 2007). In contrast to the ERP findings there were no group or treatment effects on this measure, which is not uncommon (although cf. Van Honk et al., 2005). Previous studies have reported significant effects on early ERPs in the absence of behavioral effects with supraliminal (Kolassa & Miltner, 2006; van Peer et al., 2010) as well as subliminal task versions (van Peer et al., 2010). It has been suggested that reaction times in the Emotional Stroop

paradigm result from later processes than attentional capture (see e.g., Bar-Haim et al., 2007), which can explain why they do not reflect the changes in early automatic processing that we found in our ERP measures.

Finally, a few limitations of this study should be discussed. First, as is common in testosterone administration studies, only female participants were tested because the parameters for neurophysiological effects of a single dose of testosterone cyclodextrin in men are as yet unknown (Tuiten et al., 2000). Future research should investigate whether testosterone administration has similar effects in men, as is suggested by some studies showing similarities in social behavior across sexes related to both exogenous (Goetz et al., 2014) and endogenous testosterone (Van Honk et al., 1999; but cf. Maner, Miller, Schmidt, & Eckel, 2008 for gender differences in testosterone responses to dominance threat in socially anxious men and women). Second, as we used exogenous administration, our results provide insight in the causal influence of testosterone on cognitive-emotional processes that play an important role in social behavior, and are relevant for social anxiety disorder. However, it should be noted that the results of this study cannot simply be generalized to naturalistic situations with elevated testosterone levels. Further research is needed to assess the ecological validity of our findings by comparing them with the effects of endogenous testosterone increases. Third, we used a subliminal version of the Emotional Stroop task, as previous studies suggested that effects of testosterone are more pronounced for preconscious processing of threat (Van Honk et al., 2000, 2005). However, the backward masking assumedly prevented further conscious or controlled processing of the stimuli (Van Honk et al., 2000; van Peer et al., 2010), which may explain the absence of ERP effects in later processing stages. Third, not all socially anxious participants met the criteria for a clinical diagnosis of generalized SAD at the time of testing, and the groups were relatively small, which may have attenuated the power to detect group differences. Finally, the electrode configuration used did not allow us to include electrodes at positions P7 and P8 of the extended 10-20 electrode system, which are the sites where the N170 amplitude is typically maximal. This might be an

alternative explanation for why we did not find valence or group differences in amplitude (GFP) at the time of the N170 peak.

In conclusion, our findings suggest that testosterone changed the initial basic face perception process for neutral faces, and decreased a subsequent attentional bias for social threat, in socially anxious and non-anxious participants. These findings indicate that testosterone specifically affects the early automatic processing of social cues, and provides support for the notion that testosterone affects biologically prepared motivational processes (e.g., Radke et al., 2015; Van Honk et al., 2005), which may be key to changes in social motivational behavior, such as decreased threat avoidance.

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Conflict of interest

The authors declare no conflicts of interest.

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Figure 1

Results of the T-AAHC Segmentation Procedure

Note. a) and b) The sequence of 10 distinct topography solutions are shown under GFP curves for both groups (HC left, SAD right) and all experimental conditions. Each color (and number) labels a period of spatially distinct and temporally stable electric field topography. Time 0 indicates onset of the face stimulus (16.7 ms) followed by a mask. Please note that these grand average data are not the final result. A second step (fitting the individual data) is needed to define the microstates that remain statistically significant and differ between conditions. c) Topographical properties of the 10 maps, with colors and numbers corresponding to the segmentation in panel a and b. Maps are oriented with the nasion upward and left scalp leftward. Blue denotes negative and red positive scalp potentials.

The timing and topography of these maps suggest they correspond to the C1 (map #1, <80 ms), P1 (map #2, 80-120 ms), N170/VPP (map #3 and #4, 120-200 ms), occipital P2 (map #5 and #6, 180-300 ms), and P3/LPP (maps #7-10, 275-800 ms) ERP components, respectively.

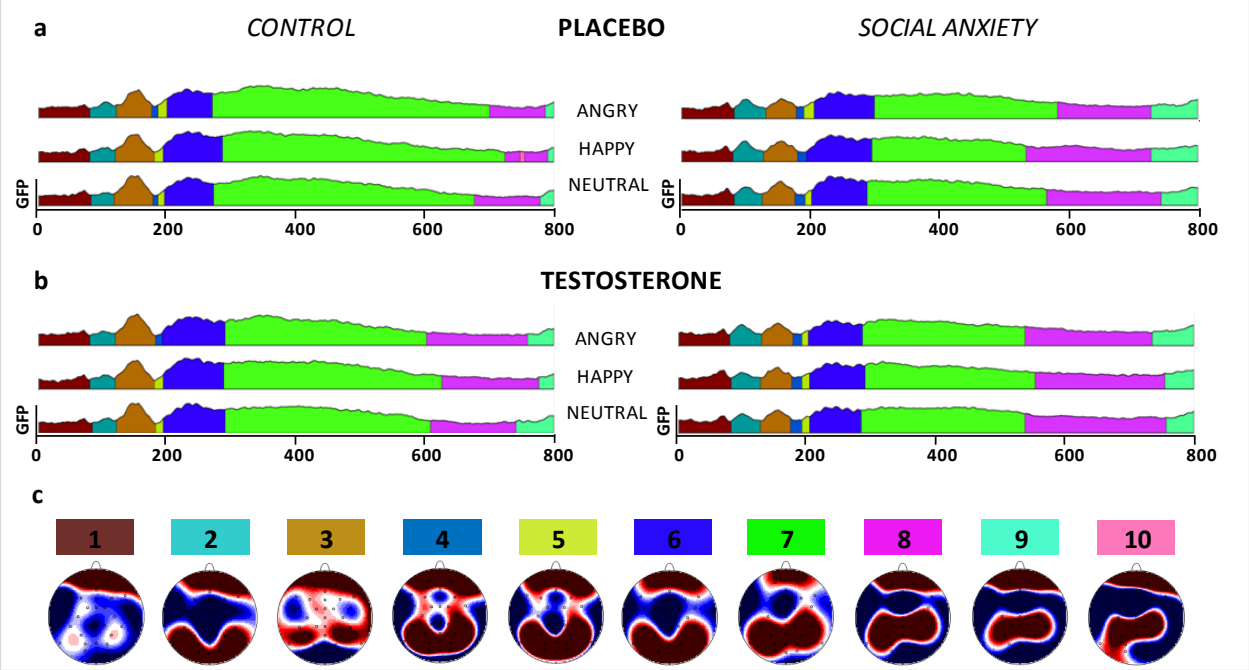

Figure 2

Results of the Fitting Procedure

Note. Global Explained Variance of map #3 and map #4 from 120-200 ms post-stimulus (corresponding to the time window of the N170/VPP) for the different experimental conditions, averaged over all participants. Results showed a significant interaction of Treatment x Valence x Map. *p<.05

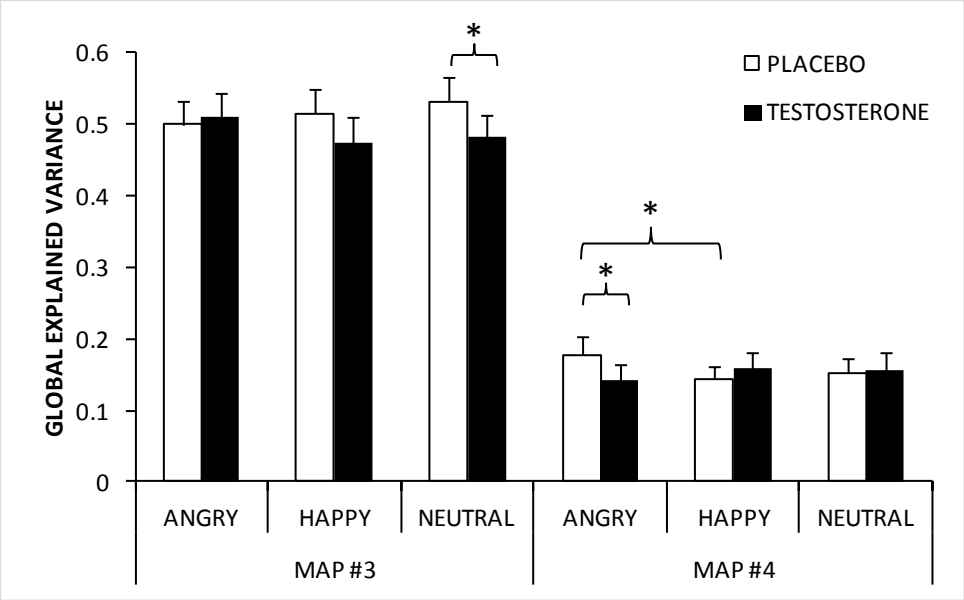

Figure 3

Global Field Power (GFP) by Group

Note. Values are averaged per Group (HC, Healthy Controls, *n*=18; SAD, Social Anxiety Disorder, *n*=18) over all experimental conditions (Treatment x Valence). Boxes indicate the time windows used for computing the averages for each ERP component: 80-120 ms (P1), 125-185 ms (N170/VPP), and 215-275 ms (P2) post-stimulus.

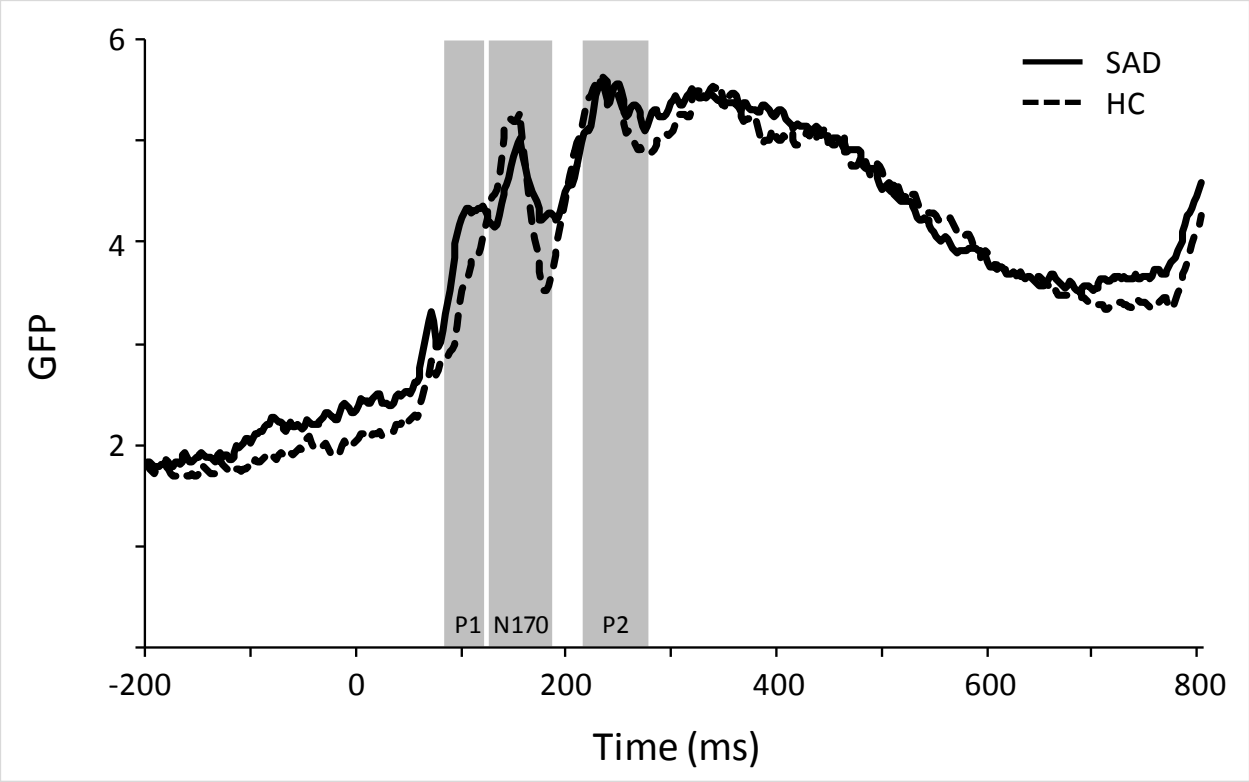

Table 1

Group Characteristics

Variable	HC (*n*=19)	SAD (*n*=19)	*p*
Order (testosterone first)	*n* = 8	*n* =11	.330
Age	25.3 (4.1)	23.0 (4.5)	.104
LSAS social anxiety	9.4 (7.1)	43.2 (6.7)	<.001
LSAS avoidance	7.7 (6.2)	37.0 (7.8)	<.001
LSAS total	17.1 (12.8)	80.2 (13.5)	<.001
SPAI social phobia	47.6 (26.6)	122.6 (23.4)	<.001
SPAI agoraphobia	10.9 (10.1)	23.5 (10.2)	.001
SPAI difference	36.7 (22.2)	99.2 (21.5)	<.001
BDI	2.5 (2.2)	14.7 (11.9)	<.001

Note. Data are presented in mean and standard deviation. Abbreviations: HC, Healthy Controls; SAD, Social Anxiety Disorder; LSAS, Liebowitz Social Anxiety Scale; SPAI, Social Phobia and Anxiety Inventory; BDI, Beck Depression Inventory. *P*-values indicate group differences.

Table 2

Mean (and SEM) Color Naming Latencies (in ms)

	HC (*n* = 19)		SAD (*n*=19)	
Valence	Placebo	Testosterone	Placebo	Testosterone
Angry	458 (14)	451 (15)	453 (14)	442 (15)
Happy	461 (15)	457 (16)	448 (15)	447 (16)
Neutral	451 (13)	435 (14)	448 (13)	437 (14)

Table 3

Means (and SEM) of the Global Field Power

		HC (*n*=18)		SAD (*n*=18)	
Time window	Valence	Placebo	Testosterone	Placebo	Testosterone
80-120 ms (P1)	Angry	3.9 (0.2)	4.1 (0.2)	3.3 (0.2)	3.5 (0.2)
	Happy	4.0 (0.2)	3.9 (0.2)	3.4 (0.2)	3.4 (0.2)
	Neutral	4.1 (0.3)	3.8 (0.2)	3.3 (0.3)	3.5 (0.2)
125-185 ms (N170/VPP)	Angry	4.5 (0.3)	4.5 (0.4)	4.4 (0.3)	4.6 (0.4)
	Happy	4.6 (0.4)	4.5 (0.4)	4.4 (0.4)	4.5 (0.4)
	Neutral	4.6 (0.4)	4.4 (0.4)	4.5 (0.4)	4.6 (0.4)
215-275 ms (P2)	Angry	5.3 (0.5)	5.3 (0.5)	5.1 (0.5)	5.4 (0.5)
	Happy	5.3 (0.6)	5.5 (0.6)	5.3 (0.6)	5.5 (0.6)
	Neutral	5.6 (0.6)	5.2 (0.5)	5.1 (0.6)	5.5 (0.5)